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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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PEPPER HAMILTON
ONE MELLON CENTER, 50TH FLOOR
500 GRANT STREET
PITTSBURGH, PA 15219

EXAMINER
NGUYEN, QUANG

ART UNIT	PAPER NUMBER
1636	14

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Please find below and/or attached an Office communication concerning this application or proceeding.

Offic Action Summary	Application No. 09/487,318	Applicant(s) REID ET AL.	
	Examiner Quang Nguyen	Art Unit 1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *07 January 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-9,11-35,38,39 and 42-46* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-9,11-35,38,39 and 42-46* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

The request filed on January 07, 2002 for a Continued Prosecution Application (CPA) under 37 CFR 1.53(d) based on parent Application No. 09/487,318 is acceptable and a CPA has been established. An action on the CPA follows.

Claims 1-9, 11-35, 38-39 and 42-46 are pending in the present application, and they are examined on the merits herein.

Sequence compliance

The disclosure is objected to because of the following informalities: The specification contains sequence listings. The nucleotide sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 C.F.R. 1.821 - 1.825 (See page 46). It should be noted that for any nucleotide sequence longer than 9 nucleic acid residues or any peptide sequence longer than 3 amino acid residues, a SEQ ID NO must be assigned to each nucleotide or peptide sequence. Applicant's attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990). If the effective filing date is on or after July 1, 1998, see the final rulemaking notice published at 63 FR 29620 (June 1, 1998) and 1211 OG 82 (June 23, 1998).

Appropriate correction is required.

N w Matter

Newly added claim 45 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Claim 45 recites "the method of claim 3 in which the immature cells have a diameter greater than about 5 microns". There is literal no support in the originally filed specification for retaining immature cells have a diameter greater than about 5 microns. The specification teaches the use of hepatic progenitors of 7-15 microns for the instant invention (page 13, line 25). Therefore, given the lack of guidance provided by the originally filed specification, it would appear that Applicants did not contemplate or have possession of the claimed invention at the time the application was filed.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-9, 12-19, 45-46, 27-35 and 39 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claims 1-9, 12-19 and 45-46 are directed to methods of providing a composition of a mixture of cells derived from human liver tissue or an enriched population of human

progenitors. To the extent that the methods are intended for providing the compositions of the presently claimed invention to a host, when read in light of the specification the sole intended purpose for such methods is for treatment of the host.

Claims 27-34 are directed to a method of treating liver dysfunction or disease responsive to treatment with liver progenitors in a subject in need thereof, comprising administering to the subject an effective amount of human liver progenitors, their progeny, more mature forms thereof, or combinations thereof, in a pharmaceutically acceptable carrier and treating the liver dysfunction or disease.

Claim 35 is directed to a method of treating a disease in a subject in need thereof comprising administering an effective amount of human hepatic progenitors, their progeny, or more mature forms thereof in which the human hepatic progenitors, their progeny, or more mature forms harbor exogenous nucleic acid.

Claim 39 is drawn to a pharmaceutical composition comprising an enriched population of human liver progenitors, their progeny, or more mature forms thereof, which human liver progenitors exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both; and a pharmaceutically acceptable carrier.

The specification teaches by exemplification the isolation of human liver progenitor cells from fetal and adult human livers. With regard to the nature of the instant claims, example 10 of the specification discloses that hepatic damage in C57BL/6 mice was induced by perfusion of hepatic progenitors infected with recombinant adenovirus expressing human urokinase plasminogen activator (uPA) via the portal vein. By day 3, treated mice had a moderate inflammatory infiltrate

comprising macrophages and neutrophils, and degenerative changes in hepatocytes were observed. Eight to 10 days after the transplantation of the infected hepatic progenitors, it was reported that there was evidence of hepatic recovery (e.g., presence of multifocal regeneration, heterologous size of nucleic, decreased inflammatory reactions with few degenerative hepatocytes). It was suggested that the urokinase expression in combination with hepatic progenitors induced significant liver parenchymal cell regeneration. The above evidence has been noted and considered. However, it is not reasonably extrapolated to the instant claimed invention.

The specification is not enabled for the instant claimed invention because it fails to provide sufficient teachings and guidance demonstrating that by administering human liver progenitors of the present application into a host or a subject having any liver dysfunction or disease, the subject would be treated for symptoms associated with the liver dysfunction or disease. At the effective filing date of the present application, the art on transplantation of liver progenitors for treating liver dysfunction or disease was still immature and highly unpredictable, particularly for attaining the desired therapeutic effects (Shafritz, Hepatology, pages 1399-1400, 2000; IDS; Vessey et al., Pathology 33:130-141, 2001; see the section entitled "Future applications of hepatic stem cells"). Shafritz stated that "...liver transplantation is the only available current therapy for end-stage liver failure.....finding alternative methods for liver replacement is of utmost importance. One such method would be functional repopulation of the diseased liver by cell transplantation." (page 1399, column 1, lines 1-6). Thus, it is apparent that a method for treating any and all liver dysfunction in a subject using cell transplantation,

including human liver progenitors of the instant invention, is not achievable or routine or predictable even in the year 2000, let alone at the effective filing date of the present application (January 19, 1999). Additionally, Shafritz et al. further noted that "the clonality and bipotent nature of isolated liver stem/progentior cells need to be confirmed by *in vivo* transplantation studies" because there is no consistent data in the art (page 1400, col. 1, middle paragraph, lines 12-26). Furthermore, there is no apparent correlation between an increase in the uptake of hepatic 3H-thymidine in mice treated with hepatic progenitors infected with recombinant adenovirus expressing human urokinase plasminogen activator (uPA) to obtaining any therapeutic effects contemplated by the treatment methods claimed. There is also no evidence of record indicating that the transplanted hepatic progenitors give rise directly to regeneration of parenchymal cells, and not due to the proliferation of endogenous hepatocytes. Thus, there is a lack of a nexus between a specific given example provided by the specification and the methods as claimed. In addition, the specification fails to provide specific relevant information such as the effective cell dosages, the frequency of administration and the exact site of introduction for a given specific liver dysfunction or disease to obtain any therapeutic effects contemplated by Applicants. The instant claims encompass autologous, allogeneic as well as xenogenic transplantation of human liver progenitors into a subject having liver dysfunction or disease. There is no evidence indicating that that the delivered human liver progenitors, their progeny or mature forms thereof would be free from the adverse host immune reactions. It is already well known in the art that adverse host immune rejection reactions present a

formidable challenge in the transplantation of allogeneic and particularly xenogeneic cells and tissues. There is no evidence of record indicating that the newly introduced human liver progenitors would be properly engrafted, proliferated, and differentiated into mature and functional liver cells or any mature functional cells derived from human liver progenitors to yield the desired therapeutic effects in any treated subject. Even in the exemplified example, moderate inflammatory responses were noted and persisted for 3 to 4 weeks in the mouse model, and there was no evidence indicating that the administered liver progenitors proliferate, differentiate into functional liver cells. The instant claims also encompass any administering routes for the delivery of liver progenitors into a subject having a liver dysfunction or disease. However, it is unclear whether effective levels of liver progenitors can home in to a diseased or disordered liver to proliferate and differentiate into functional mature cells (liver cells or any mature cells derived from human liver progenitors) to yield the desired therapeutic effects. Moreover, Dabeva et al. (Am. J. Pathology 156:2017-2031, 2000; IDS) have stated that "Several studies also report successful engraftment and differentiation of early fetal liver tissue or cell suspensions after transplantation into ectopic sites. However, engrafted liver tissue masses at ectopic sites do not expand very much, and it is unlikely that such limited liver transplantation will have broad therapeutic application" (page 2029, column 2, last 3 sentences continue to lines 1-4 of column 1, page 2030). The mere mentioning of advantages offered by human liver progenitors for *ex vivo* gene therapy and routes of tranplantation (See specification, pages 40-44) is not seen as providing enablement as there is no correlation between these and any therapeutic outcome. Without the

specific teaching or guidance provided by the specification, it would have required undue experimentation for one skilled in the art to make and use the instant claimed invention, particularly in light of the state of the art of human liver progenitor cell transplantation therapy. The CAFC has stated that "patent protection is granted in return for an enabling disclosure, not for vague intimations of general ideas that may or may be workable". The court continues to state that "tossing out the mere germ of an idea does not constitute an enabling disclosure" and that "the specification, not knowledge in the art, that must supply the novel aspects of an invention in order to constitute adequate enablement". (See *Genetech, Inc. v. Novo Nordisk A/S*, 42 USPQ 2d 1001, at 1005).

With regard to the breadth of claim 35 encompassing human hepatic progenitors, their progeny, or more mature forms thereof comprising any and all exogenous nucleic acid to treat any disease in a subject, the specification fails to teach any specific vector used to deliver and express a specific gene (a therapeutic protein) in human hepatic progenitor cell populations of the instant claimed invention for treating a specific disease. In addition to the obstacles of a human liver progenitor cell transplantation therapy discussed in the preceding paragraphs, at the effective filing date of the present application, it has been noted that sub-optimal vectors, the lack of long-term and stable transgene expression are some of the factors limiting an effective gene therapy. In a review on gene delivery systems available for gene therapy (Methods of gene delivery, Hematol. Oncol. Clin. North Am. 12:483-501, 1998), Wivel and Wilson stated that "One of the major challenges still confronting the field is the design of more efficient vectors.

The gene delivery systems being used today will undoubtedly be seen as crude when compared with future developments. It is unlikely that there will ever be a universal vector, but rather there will be multiple vectors specifically designed for certain organ sites and certain diseases... It will be necessary to do much more fundamental research in cell biology, virology, immunology, and pathophysiology before vectors can be significantly improved." (pages 498-499 in Summary section). On the basis of the instant disclosure, there is no evidence of record that human hepatic progenitors, their progeny or more mature forms thereof haboring exogenous nucleic acid could persist in a treated subject for a sufficient period of time to provide a stable, long-term *in vivo* expression of therapeutic transgenes to yield the therapeutic outcomes for any and all diseases. As written, the claims do not even require any expression of any therapeutic genes since the cells merely harbor exogenous nucleic acid which is not necessarily coupled to any promoter or enhancer. *In vivo* expression of therapeutic transgenes is known to be transient. This is supported by numerous teachings in the art. As examples, Palmer et al. (Proc. Natl. Acad. Sci. 88:1330-1334, 1991) demonstrated that the *in vivo* expression of human factor IX by transplanted syngeneic recombinant fibroblasts was transient and vanished 1-5 weeks post-transplantation. Riddell et al. (Nature Med. 2:216-223, 1996) reported that five out of six patients seropositive for human immunodeficiency virus quickly developed cytotoxic T-lymphocytes responses specific to a novel protein and eliminated infused autologous CD8+ HIV-specific cytotoxic T cells transduced with a fusion suicide gene (See abstract). Additionally, factors such as the level of mRNA produced, the stability of the protein produced, the

protein's compartmentalization within the cell or its secretory fate differ dramatically based on which protein being produced, and therefore the desirable therapeutic effect sought to achieve. Thus, the level of gene expression, its duration, and its *in vivo* therapeutic effects are not always predictable, and they can not be overcome with routine experimentation. Accordingly, with the lack of guidance provided by the instant specification, it would have required undue experimentation for one skilled in the art to make and use the method as claimed.

With respect to claim 39 directed to a pharmaceutical composition comprising an enriched population of human liver progenitors, their progeny or more mature forms thereof, the instant specification is not enabled for the use of said composition to treat liver dysfunction or disease for the same reasons discussed above. It is noted that enablement requires the specification to teach how to make and use the claimed invention.

Accordingly, due to the lack of direction or guidance presented in the specification regarding to the issues set forth above, the unpredictability of the human liver progenitor cell transplantation therapy and gene therapy arts, the absence of working examples, and the breadth of the claims, it would have required undue experimentation for one skilled in the art to make/use the claimed invention.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on April 16, 2001 in Paper No. 9 (pages 4-6) have been fully considered.

Applicants basically argued that the instant specification provides sufficient guidance for the use of the isolated progenitors of the invention and their progeny for the treatment of liver diseases and dysfunctions as claimed by referring to various passages in the application. Applicants maintained that the administration of suspensions of hepatocytes to subjects is routine, and that Applicants discussed the advantages hepatic progenitors versus hepatocytes with regard to cell size, immunological rejection and proliferative capacity for treatment purposes. Applicants also maintained that "Once the hepatic progenitors are administered they then go about the business of alleviating the medical condition affecting the liver". Additionally, Applicants cited the case Hybritech vs. Monoclonal Antibodies, Inc. to point out that the level of precision need only be as precise as the art can offer. Applicants further cited the reference of Halibullah reporting the use of hepatocytes for treatment of fulminant liver failure by simple injection into the peritoneum to establish the general validity and ease of cell therapy with liver-derived cells. Examiner respectfully finds Applicants' arguments to be unpersuasive for the following reasons.

The mere general description or mentioning the advantages offered by human liver progenitors for treating a host of liver dysfunction, disorders and diseases such as hepatocholangitis, cirrhosis, hepatitis, acute and chronic liver failure, hepatocarcinoma,

hepatoblastoma and others, is not seen as providing enablement as there is no correlation between these general descriptions and contemplated therapeutic outcomes. Without the specific guidance provided by the specification, it would have required undue experimentation for one skilled in the art to make and use the methods as claimed. It should be noted that the physiological art is recognized as unpredictable (MPEP 2164.03), especially for achieving therapeutic effects for a whole host of liver diseases and disorders as claimed. In this regard, the cited case law of Hybridtech vs. Monoclonal Antibodies, Inc. concerning antibodies affinities is not applicable. In contrary to Applicants' simple assertion that once the hepatic progenitors are administered into a subject suffering from a liver dysfunction or disease, they then go about the business of alleviating the medical condition affecting the liver, there are several issues that the specification fails to address such that contemplated therapeutic outcomes could be attained. As noted in the previous Office Action, there is no evidence indicating that the introduced human liver progenitors are properly engrafted, proliferated, and differentiated into mature and functional liver cells in any treated subject to yield any therapeutic outcomes or effects. As recited, the claims encompass syngeneic, allogeneic and xenogenic transplantation of human liver progenitors into a subject in need of treatment. There is no evidence indicating that that the delivered human liver progenitors, their progeny or mature forms thereof would be free from the adverse host immune reactions. It is already well known in the art that adverse host immune rejection reactions present a formidable challenge in the transplantation of allogeneic and particularly xenogeneic cells and tissues. Even in the exemplified

example, moderate inflammatory responses were noted and persisted for 3 to 4 weeks in the mouse model, and there is no evidence indicating that the administered liver progenitors proliferate, differentiate into functional liver cells. The instant claims also encompass any and all administering sites for the delivery of liver progenitors into a subject having a liver dysfunction or disease. However, it is unclear whether effective levels of liver progenitors can home in to a diseased or disordered liver to proliferate and differentiate into functional mature cells to yield therapeutic effects by any and all routes of administration. Moreover, Dabeva et al. (Am. J. Pathology 156:2017-2031, 2000; IDS) have stated that "Several studies also report successful engraftment and differentiation of early fetal liver tissue or cell suspensions after transplantation into ectopic sites. However, engrafted liver tissue masses at ectopic sites do not expand very much, and it is unlikely that such limited liver transplantation will have broad therapeutic application" (page 2029, column 2, last 3 sentences continue to lines 1-4 of column 1, page 2030). With regard to the reference of Habibullah et al. (Transplantation 58:951-977, 1994, IDS), as rightly pointed out by Applicants that the treatment with hepatocyte in the study of Habibullah et al. is not the same as treatment with liver progenitors, for one thing the starting material is not the same. Therefore, the positive therapeutic effects observed from the study of Habibullah et al. could not be reasonably extrapolated to the contemplated therapeutic effects to be achieved by the instant invention. Furthermore, it should be noted in the study of Habibullah et al. the beneficial effects were limited specifically to 2 patients having PSE of grade III and 1 patient having PSE of grade IVa, and that the selected patients have fulminant hepatic

failure (FHF) of less than two weeks of duration. Additionally, it is unclear about the fate of the transplanted fetal hepatocytes. Whether they could be stably engrafted in the peritoneum, continued to proliferate and provided beneficial effects for the patients. Habibullah et al. also noted that in rat and rabbit models of fulminant hepatic failure, perioteneal survival of transplanted syngeneic hepatocytes lasted only about 10 days (page 352, column 1, full paragraph, lines 3-6). Because of the very limited beneficial effects observed, specifically for 3 patients with PSE of grade III and IVa in a very small selected patient population (7 patients having FHF less than two weeks of duration), the results of Habibullah et al. certainly can not be reasonably extrapolated to the therapeutic results hoped to be achieved for treating any and all liver dysfunction and diseases in a subject as encompassed by the breadth of the instant claims. Lastly, in a recent review on liver stem cell, Shafritz (Hepatology, pages 1399-1400, 2000; IDS) stated that "...liver transplantation is the only available current therapy for end-stage liver failure.....finding alternative methods for liver replacement is of utmost importance. One such method would be functional repopulation of the diseased liver by cell transplantation." (page 1399, column 1, lines 1-6). So clearly, a method for treating any and all liver dysfunction in a mammal using cell transplantation, including human liver progenitors of the instant invention, is not achievable or routine or predictable even in the year 2000, let alone at the effective filing date of the present application (January 19, 1999). Interestingly, Shafritz et al. further noted that "the clonality and bipotent nature of isolated liver stem/progentior cells need to be confirmed by *in vivo* transplantation studies" because there is no consistent data in the art (page 1400, col.

1, middle paragraph, lines 12-26). Accordingly, given the lack of provided by the instant specification, the unpredictability of the physiological art, the state of liver progenitor cell transplantation, and the breadth of the instant claims, it would therefore have required undue experimentation for a skilled artisan to make and use the methods as claimed.

With regard to claim 35, Applicants argued that the specification provides sufficient guidance regarding to providing hepatic precursors with an exogenous gene of interest for treatment purposes by citing various passages in the specification and example 10. Applicants further argued that it is not necessary to give an example of every species. Although Examiner agrees with Applicants the latter point, however, Applicants' arguments are found to be unpersuasive. This is again because the general description in the specification is not deemed to be equivalent to the therapeutic outcomes contemplated by the method as claimed. Furthermore, the specification is not enabled for the claimed method for the reasons stated in the preceding paragraphs. Additionally, there are issues such as whether the genetically modified human hepatic progenitors, their progeny or mature forms thereof could be stably engrafted, differentiated and proliferated into functional liver cells could be achieved in the treated subject and whether said cells persist in a treated subject for a sufficient period of time to provide a stable, long-term *in vivo* expression of therapeutic transgenes to yield therapeutic outcomes for any and all diseases. The nature of the claim 35 falls within the realm of *ex vivo* gene therapy which was highly unpredictable at the effective filing date of the present application with respect to achieving therapeutic results. As noted in the previous Office Action, factors such as sub-optimal vectors, the lack of a long-term

and stable *in vivo* transgene expression are some of the factors limiting the effectiveness of *ex vivo* gene therapy. For examples, Palmer et al. (Proc. Natl. Acad. Sci. 88:1330-1334, 1991) demonstrated that the *in vivo* expression of human factor IX by transplanted syngeneic recombinant fibroblasts was transient and vanished 1-5 weeks post-transplantation. Riddell et al. (Nature Med. 2:216-223, 1996) reported that five out of six patients seropositive for human immunodeficiency virus quickly developed cytotoxic T-lymphocytes responses specific to a novel protein and eliminated infused autologous CD8+ HIV-specific cytotoxic T cells transduced with a fusion suicide gene (See abstract). Given the unpredictability of the gene therapy art coupled with the lack of specific guidance provided by the instant specification with respect to attaining therapeutic effects for any and all disease in a host by overcoming the aforementioned factors, it would have required undue experimentation without a predictable expectation of success for one skilled artisan to make and use the instant claimed invention.

Accordingly, claims 1-9, 12-19, 45-46, 27-35 and 39 are rejected under 35 U.S.C. 112, first paragraph for the reasons set forth above.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-9, 11-20, 27-35 and 45-46 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The terms "substantially", "relatively large size" and "relatively small size" in claim 1 are relative terms that render the claim indefinite. The terms "substantially", "relatively large size" and "relatively small size" are not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Clarification is requested.

In claims 1, 12 and their dependent claims, it is unclear what is encompassed by the phrase "A method of providing a composition" recited in the preamble of the claims. Providing a composition to a host or what? Or do Applicants intend to claim methods for preparing the compositions of the presently claimed invention? The metes and bounds of the claims can not be clearly determined. Clarification is requested. If Applicants intend to claim a method of providing a composition to a host or a patient, then the claims are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted step is the providing step to a host or a patient as intended by Applicants.

In claims 35, 27 and its dependent claims, it is unclear what is encompassed by the phrase "in a subject in need thereof". Based on which criteria or conditions that a subject is in need of the treatment with liver progenitors or not? The metes and bounds of the claims can not be clearly determined.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on April 16, 2001 in Paper No. 9 (pages 6-7) have been fully considered.

Applicants argued that the meaning of "relatively large size" and "relatively small size" is well established in the disclosure of the invention and will be clear to one skilled in the art upon examination of the specification by referring to page 26, line 30 and page 27, line 12 as examples. Examiner respectfully finds Applicants' argument to be unpersuasive because the terms are not clearly defined in the specification and therefore the metes and bounds of the claims are still not exactly determined. If Applicants intend to remove cells larger than 15 microns in diameter while retaining cells less than 15 microns in the claimed method, then please recite the claims as such. Although Applicants removed the term "substantially single" in amended claim 1 as suggested by Examiner, the same term is still present in the amended independent claim 12.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

The changes made to 35 U.S.C. 102(e) by the American Inventors Protection Act of 1999 (AIPA) do not apply to the examination of this application as the application being examined was not (1) filed on or after November 29, 2000, or (2) voluntarily published under 35 U.S.C. 122(b). Therefore, this application is examined under 35 U.S.C. 102(e) prior to the amendment by the AIPA (pre-AIPA 35 U.S.C. 102(e)).

Claims 12-19 are rejected under 35 U.S.C. 102(b) as being anticipated by Muench et al. (Blood 83:3170-3181, 1994) or Muench et al. (Blood 89:1364-1375, 1997) for the same reasons set forth in the previous Office Action mailed on 07/05/01 in Paper No. 11.

The claims are drawn to a method of preparing a composition comprising an enriched population of human liver progenitors comprising: (a) providing a substantially single cell suspension of human liver tissue, and (b) subjecting the suspension to a positive or negative immunoselection, such that a mixture of cells is provided, which mixture of cells is comprised of an enriched population of human liver progenitors, which human liver progenitors themselves, their progeny, or more mature forms therof exhibit one or more markers indicative of expression of alpha-fetoprotein. Claim 20 is directed to a human liver progenitor isolated by the same method.

Muench et al. (1994, 1997) disclosed a method for the isolation of human fetal liver progenitors and hematopoitic stem cells derived from human fetal liver. The method comprises subjecting a suspension of fetal liver cells to a density centrifugation to obtain light density fetal liver (LDFL) cells (See column 1, page 3171, Muench et al., 1994; column 2, first paragraph, page 1365, Muench et al., 1997). LDFL cells were

depleted of glycophorin A (GPA^-) cells by immunomagnetic beads depletion (a form of negative immunoselection), then GPA^- LDFL cells were enriched for $CD34^+$ cells by panning using an anti-CD34 antibody-coated tissue culture flasks. GPA^- LDFL cells were also fractionated based on cell-surface antigen expression by FACS (See column 1, page 3171, Muench et al., 1994; column 2, first paragraph, page 1365, Muench et al., 1997). As the method of Muench et al. (1994, 1997) and the method of the instant claims are not distinguishable, the method of Muench et al. inherently produces an enriched population of human liver progenitor cells as claimed. Therefore, the references anticipate the claimed invention.

Claims 21-23 and 42-44 are rejected under 35 U.S.C. 102(b) as being anticipated by Muench et al. (Blood 83:3170-3181, 1994) or Muench et al. (Blood 89:1364-1375, 1997) for the same reasons set forth in the previous Office Action mailed on 07/05/01 in Paper No. 11.

Claims 21-23 are drawn to a composition comprising an enriched population of human liver progenitors, their progeny, or more mature forms thereof, which human liver progenitors exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both; the same composition wherein the progenitors comprise hepatic progenitors, hematopoietic progenitors, mesenchymal progenitors, or combinations thereof, and the same wherein said human liver progenitors, their progeny, or more mature forms thereof express CD14, CD34, CD38, CD117, ICAM or combinations thereof. Claim 42 is directed to isolated human liver progenitors, their progeny or more

mature forms whereof which exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both. Claims 43-44 are drawn to isolated human liver progenitors, their progeny or more mature forms thereof which exhibit the phenotype glycophorin A^-, $CD45^-$, alpha-$fetoprotein^{+++}$, $albumin^+$, and $ICAM^+$, and the same which further express $CD14^+$, $CD34^{++}$, $CD38^{++}$, $CD117^{+++}$, or combinations thereof.

Muench et al. (1994) disclosed the isolation of human fetal liver progenitors with a high proliferative potential and a phenotype of $CD34^+$, $CD33^+$, $CD13^+$, CD38-, lin^- (lineage= CD3, CD8, CD10, CD14, CD15, CD16, CD19, CD20 and glycophorin A), $CD45RA^-$, $CD45RO^-$, $CD71^-$, and heterogeneous *for c-kit* or CD117 (See abstract and page 3171). Muench et al. (1997) disclosed the isolation of hematopoietic stem cells derived from human fetal liver, with a phenotype of $CD4^+$, $CD34^{++}$, Lin^-, $CD117^+$, $CD38^-$, $CD45RA^-$ (See abstract and page 1365). As the method of Muench et al. (1994, 1997) and the method of the presently claimed invention are not distinguishable, the method of Muench et al. inherently produces an enriched population of human liver progenitor cells as claimed. Therefore, the references anticipate the claimed invention.

Claims 12-15, 18-23, and 42-44 are rejected under 35 U.S.C. 102(b) as being anticipated by Craig et al. (J. Exp. Med. 17:1331-1342, 1993) for the same reasons set forth in the previous Office Action mailed on 07/05/01 in Paper No. 11. To the extent that the method of the instant claims is interpreted as a method for preparing a composition comprising a mixture of cells derived from human liver tissue, the following rejection is applied.

Craig et al. disclosed the isolation of human hematopoietic progenitor cells derived from human fetal liver with a phenotype of Thy-1^{+}, CD34^{+}, CD38^{low}, CD45RA^{-}, CD45RO^{+}, CD71^{low}, and CD117^{low} (See abstract, and column 1, second paragraph, page 1332). The method comprises the preparation of low density mononuclear cells by density centrifugation using Ficoll-Paque (column 1, page 1332, lines 24-26). In some samples, red blood cells were lysed by the addition of 10-fold excess of ammonium chloride lysing solution (column 1, page 1332, lines 30-32). Subpopulations of low density mononuclear cells were subsequently sorted by multiparameter flow cytometry, a form of positive immunoselection (column 2, page 1335, second paragraph). As the method of Craig et al. (1993) and the method of the instant claims can not be distinguished, the method of Craig et al. inherently produces an enriched population of human liver progenitor cells as claimed. Thus, the reference anticipates the claimed invention.

Claims 11, 20, 21-26 and 42-44 rejected under 35 U.S.C. 102(e) as being anticipated by Faris (U.S. Patent No. 6,129,911 with an effective filing date of 7/10/1998) for the same reasons set forth in the previous Office Action mailed on 07/05/01 in Paper No. 11.

Claims 21-26 are drawn to a composition comprising an enriched population of human liver progenitors, their progeny, or more mature forms thereof, which human liver progenitors exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both; the same composition in which progenitors habor exogenous nucleic

acid promoting the expression of at least one polypeptide of interest. Claims 11 and 20 are directed to a human liver progenitor isolated by the methods of claims 1 and 14, respectively. Claims 41-43 are drawn to isolated human liver progenitors encompassing hepatic progenitors.

Faris taught the preparation and isolation of a liver cell cluster of less than 10 cells comprising a liver stem cell and a hepatocyte, and a primary liver stem cell derived from human liver tissue, in which said stem cell comprises DNA encoding a heterologous polypeptide, such as ornithine transcarbamylase, glutamine synthetase, Factor XIII, Factor IX and others (See columns 1-3, and the claims). The primary liver stem cell derived from human liver tissue is defined as undifferentiated cell that differentiates into a mature functional hepatocyte or bile duct cell (column 1, lines 37-39) which is consistent with the definition of hepatic progenitors of the instant claimed invention (cells give rise to hepatocytes and biliary cells, page 22, lines 3-4). Since a product and its properties can not be separated, the composition of isolated liver cell cluster of Faris is the same as an enriched population of human liver progenitors, their progeny or more mature forms thereof, or isolated human liver progenitors of the same instant invention, regardless how they are isolated, the reference therefore anticipates the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-6, 8, 12-19 and 45-46 are rejected under 35 U.S.C. 103(a) as being unpatentable over Reid et al. (U.S. Patent No. 6,069,005) in view of Naughton et al. (U.S. Patent No. 5,559,022; IDS). To the extent that the method of the instant claims is interpreted as a method for preparing a composition comprising a mixture of cells derived from human liver tissue, the following rejection is applied.

Reid et al. disclosed a method of isolating hepatic progenitors from rat fetal livers utilizing panning techniques and flow cytometry on single cell suspension of liver cells (See claim 1, column 20). The method comprises the panning and fluorescence activated cell sorting of fetal liver cells using specific antibodies to remove mature hepatocytes, mature bile duct cells, endothelial cells, mesenchymal cells and hemapoietic cells for obtaining a cell population enriched for immature hepatic cell types which were subsequently separated into distinct subcategories by multiparametric

fluorescence activated cell sorting (See Examples I and II). The panning stage involves multiple steps (see Table 3 in col. 6, for example) resulting in isolated cells enriched up to 5-fold for AFP mRNA and 2-fold for albumin mRNA (col. 17, lines 19-27). One of the panning steps is a selecting step for cells exhibiting one or more markers indicative of expression of alpha-protein, albumin or both, for this instance mRNAs of AFP and albumin. Since a product and its properties can not be separated, hepatic progenitors isolated by the disclosed method also possess markers indicative of expression of alpha-fetoprotein, albumin, or both (full-length mRNAs, for examples), as well as alpha-fetoprotein-like immunoreactivity, albumin-like immunoreactivity, or a combination thereof as evidenced by the enrichment of AFP mRNA and albumin mRNA in selected cells after the panning. It is further noted that fetal liver cells selected for flow cytometry in the disclosed method have a broad range in cell size, 5 to 15 microns (See column 17, lines 50-51). Although Reid et al. did not specifically teach a method of providing a composition comprising a mixture of cells derived from human liver tissue or an enriched population of human liver progenitors, Reid et al. stated that their method offers a systematic approach to isolating hepatoblasts (hepatic progenitors) from any age from any species (column 2, lines 45-49). At the filing date of the present application, Naughton et al. teach the isolation and characterization of a liver progenitor cell population with high proliferative activity and ability to differentiate *in vitro* from human livers (see the entire patent, particularly col. 5, line 66 continues to line 4 of col. 6).

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time of invention was made to modify the method disclosed by Reid et al. by replacing rat fetal liver tissue as the starting material with human liver tissues. The motivation for one of ordinary skilled artisan to carry out the above modification is to obtain a composition enriched in a population of human liver progenitors for cellular characterization as well as for cell transplantation studies. Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 1 and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Reid et al. (U.S. Patent No. 6,069,005) in view of Naughton et al. (U.S. Patent No. 5,559,022; IDS) as applied to claims 1-6, 8, 12-19 and 45-46 above, and further in view of Craig et al. (J. Exp. Med. 17:1331-1342, 1993).

The teachings of Reid et al. and Naughton et al. have been discussed above. Neither reference teaches selective lysis of the mature cells in their isolation procedures for liver progenitor cells. However, during the isolation of human hematopoitic stem cells from fetal liver, Craig et al. teach that red blood cells were lysed by the addition of 10-fold excess of ammonium chloride lysing solution (column 1, page 1332, lines 30-32).

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time of invention was made to modify the method of Reid et al. and Naughton et al. by further incorporating a lysis step to remove mature red blood cells during the isolation of liver progenitors as taught by Craig et al. The instant claimed method is an

obvious variant of the modified method based on the combined teachings of Reid et al. and Naughton et al. Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 21 and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Muench et al. (Blood 83:3170-3181, 1994) or Muench et al. (Blood 89:1364-1375, 1997) in view of Reid et al. (U.S. Patent No. 5,789,246, PTO-1449 AB) for the same reasons set forth in the previous Office Action mailed on 07/05/01 in Paper No. 11.

The claims are drawn to a composition comprising an enriched population of human liver progenitors, their progeny, or more mature forms thereof, which human liver progenitors exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both; and a cell culture comprising the same composition, an extracellular matrix component, and a culture medium (Claim 38).

Muench et al. (1994) disclosed the isolation of human fetal liver progenitors with a high proliferative potential and a phenotype of $CD34^+$, $CD33^+$, $CD13^+$, CD38-, lin^- (lineage= CD3, CD8, CD10, CD14, CD15, CD16, CD19, CD20 and glycophorin A), $CD45RA^-$, $CD45RO^-$, $CD71^-$, and heterogeneous *for c-kit* or CD117 (See abstract and page 3171). Muench et al. (1997) disclosed the isolation of hematopoietic stem cells derived from human fetal liver, with a phenotype of $CD4^+$, $CD34^{++}$, Lin^-, $CD117^+$, $CD38^-$, $CD45RA^-$ (See abstract and page 1365). Since a product and its properties can not be separated, human fetal liver progenitors and human hematopoietic stem cells derived from human fetal liver isolated by Muenche et al. (1994, 1997) also possess the same

properties as those of the enriched population of human liver progenitor cells in the instant claimed invention. However, Muench et al. (1994, 1997) did not teach a cell culture comprising these cell populations, an extracellular matrix component, and a culture medium. However, Reid et al. taught a cell culture comprising hepatocyte precursors being plated on or in a matrix of collagen type IV and in the serum-free, hormonally defined medium (See columns 2-4) for the expansion or proliferation of hepatocyte precursors.

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time of invention was made to adopt the cell culture system taught by Reid et al. for the expansion of human fetal liver progenitors and hematopoietic stem cells derived from human fetal liver disclosed by Muench et al. (1994, 1997). One would have been motivated to carry out the above modification for expanding human fetal liver progenitors and hematopoietic stem cells for uses in artificial livers, for toxicology and pharmacology studies (See abstract in Reid et al.). Thus, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Applicants' arguments related to the above rejections in the Amendment filed on April 16, 2001 in Paper No. 9 (pages 8-10) have been fully considered.

Applicants argued with respect to amended claims, none of the cited references disclose elements of the invention. Specifically, none of the cited references teach an enriched population which exhibit one or more markers indicative of expression of

alpha-fetoprotein, albumin or both. As an example, Applicants argued that Muench et al. do not assess the expression of either alpha-fetoprotein or albumin or the correlation of expression of either protein with any marker described in Muench et al. (1994, 1997). Applicants further assert that there is no indication that the cells that Muench et al. identify inherently express either alpha-fetoprotein or albumin. Examiner respectfully finds Applicants' arguments to be unpersuasive for the following reasons. Although Examiner agrees with Applicants that the articles of Muench et al., Craig et al. and Faris do not assess the expression of either alpha-fetal protein or albumin in their cell preparations, however their cell preparations are enriched in CD34 (a common marker for progenitor or stem cells) cells, and such CD34 cells derived from human liver are known to express at least alpha-fetal protein as evidenced by the disclosure of the instant specification (page 32, Table 2; page 34, Table 3, for examples). It should be further noted that Munch et al. selected all CD34 cells derived from human fetal liver via panning using an anti-CD34 antibody. With respect to the cited reference of Reid et al., they specifically teach that the isolated hepatic progenitors after panning are enriched in AFP mRNA and albumin mRNA as noted above. Therefore, the cited references disclose elements of the present invention. Accordingly, the claims are rejected for the reasons of record.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11

F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 27-33 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 59-77 of copending Application No. 09/154224. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant claims encompass all the embodiments of the pending claims of the co-pending Application No. 09/154224.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Claim 35 is provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 21-39 of copending Application No. 09/534487. Although the conflicting claims are not identical, they are not patentably distinct from each other because a method of treating a disease in a subject in need thereof using an effective amount of human hepatic progenitors, their progeny or more mature forms thereof in which the human hepatic progenitors, their progeny, or more mature forms harbor exogenous nucleic acid of the instant application encompass the embodiments of a method of treatment of liver dysfunction in

a subject in need thereof using a genetically engineered hepatocyte precursor to the subject in the co-pending Application No. 09/534487.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Conclusions

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (703) 308-8339.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's mentor, Dave Nguyen, may be reached at (703) 305-2024, or SPE, Irem Yucel, Ph.D., at (703) 305-1998.

Any inquiry of a general nature or relating to the status of this application should be directed to Patent Analyst, Tracey Johnson, whose telephone number is (703) 305-2982.

Quang Nguyen, Ph.D.

DAVE T. NGUYEN
PRIMARY EXAMINER